Original Article

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Dentistry Section

Efficacy of Kerosene and Cedarwood Oil as Xylene Alternatives in Tissue Processing and H&E Staining: An In-vitro Study

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ABSTRACT

Introduction: Xylene is the most commonly used clearing and deparaffinising agent in histopathology laboratories. Xylene-associated health hazards include neurotoxicity, respiratory effects, and potential carcinogenicity. The literature describes several xylene substitutes used as clearing agents, such as benzene, toluene, chloroform, petroleum ether, oil of wintergreen (methyl salicylate), cedarwood oil, carbon tetrachloride, clove oil, dioxane, aniline oil, and kerosene. Commonly used alternative deparaffinising agents include toluene, Ultraclear, refined mineral oil, dishwashing liquid, coconut oil, and limonene.

Aim: To assess the efficacy of kerosene and cedarwood oil as alternative clearing and deparaffinising agents to xylene in tissue processing and Haematoxylin and Eosin (H&E) staining.

Materials and Methods: The present in-vitro study was conducted on 60 formalin-fixed tissue specimens, which were divided into three groups (A, B, and C) of 20 specimens each. All tissues underwent routine tissue processing. Alcohol-dehydrated specimens in Group A were cleared with xylene, Group B with kerosene, and Group C with cedarwood oil. After the impregnation process, tissue blocks were prepared using paraffin wax. Sections were cut, and during subsequent H&E staining, xylene was used as the deparaffinising agent for Group A, kerosene for Group B, and cedarwood oil for Group C. All slides were evaluated under a light microscope by two blinded observers for cytoplasmic staining, nuclear staining, staining contrast, and the presence of artifacts. Results were tabulated. Statistical analysis was performed using IBM Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistics, Fisher's exact test, and Chi-square tests were applied.

Results: The majority of xylene-processed tissues exhibited good cytoplasmic and nuclear staining. Kerosene-processed tissues showed equal proportions of good and fair cytoplasmic staining, with predominantly fair nuclear staining. Cedarwood oil-processed tissues displayed fair cytoplasmic and nuclear staining. Differences in cytoplasmic and nuclear staining among the groups were not statistically significant ($p=0.122$ and $p=0.127$, respectively). Most xylene- and cedarwood oil-processed tissues demonstrated good staining contrast, whereas kerosene-processed tissues predominantly showed fair staining contrast. This difference was statistically significant ($p<0.001$). Kerosene-processed tissues exhibited a higher number of artifacts, while xylene and cedarwood oil processed tissues showed fewer artifacts. This difference was also statistically significant ($p=0.002$).

Conclusion: Although kerosene is a more cost-effective and less toxic option than xylene, its inferior staining quality and higher incidence of tissue artifacts suggest that it may be suitable only after adequate purification to minimise impurities. Cedarwood oil emerges as a strong alternative to xylene, providing good staining quality with fewer artifacts, minimal tissue damage, and lower toxicity. However, its higher cost and longer processing time must be considered before routine use.

Keywords: Artifacts, Dehydrating agent, Deparaffinising agent, Haematoxylin and eosin, Tissue processing

INTRODUCTION

Microscopic examination of biopsy tissues in histopathology requires tissue processing, which involves several steps such as fixation, dehydration, clearing, impregnation, embedding, sectioning, deparaffinisation, and staining [1,2]. Tissue processing must result in adequate tissue translucency and clarity, which are essential for accurate microscopic evaluation. Therefore, clearing and deparaffinisation are crucial steps that require agents capable of ensuring tissue transparency and proper infiltration [1,2].

Various substances, including benzene, xylene, toluene, chloroform, petroleum ether, oil of wintergreen (methyl salicylate), cedarwood oil, carbon tetrachloride, clove oil, dioxane, and aniline oil, have been used as clearing agents in tissue processing. Commonly used deparaffinising agents include xylene, toluene, Ultraclear, and alternatives such as refined mineral oil, dishwashing liquid, coconut oil, and limonene [3-5].

Xylene ($(CH_3)_2CH_4$) is the most widely used clearing agent in routine histopathology worldwide. Its advantages include rapid action, suitability for most tissues, compatibility with both paraffin and celloidin embedding, and cost-effectiveness [4-6]. However, xylene must be handled with caution due to its toxic effects, primarily on the central nervous system. Acute exposure can cause headache, dizziness, nausea, and irritation of the eyes, nose, throat, and lungs. Chronic exposure may lead to memory loss, irritability, insomnia, and depression. High exposure levels may result in chest pain, breathlessness, pulmonary oedema, and reversible liver and kidney damage. Skin contact can cause dryness, cracking, dermatitis, and blistering due to the removal of natural oils [7].

Concerns regarding xylene toxicity have prompted the search for safer alternative clearing agents in histopathology. An ideal substitute should balance safety, cost-effectiveness, preservation of tissue morphology, and staining quality [7]. Kerosene and cedarwood oil have been investigated as potential alternatives, with several studies demonstrating their effectiveness in tissue processing and staining, albeit with minor procedural variations. Studies by Chaudhuri D et al., (2023), Gayathri G et al., (2016), Thamilselvan S et al., (2021), Viswasini RD et al., (2025), and Dineshshankar J et al., (2019) support their potential utility [4,7-10].

Based on these findings, the present study further investigates the suitability of kerosene and cedarwood oil as replacements for xylene in histopathological procedures. By comparing these two benzene derivatives, the current study aimed to contribute to the ongoing discussion on safer and more eco-friendly laboratory practices, thereby enhancing occupational safety and reducing reliance on hazardous chemicals such as xylene. The objective of the present study was to evaluate and compare the clearing and deparaffinising efficacy of kerosene and cedarwood oil with xylene to determine their suitability as alternatives in routine histological processing.

MATERIALS AND METHODS

The present in-vitro study was conducted at the Dental College and Hospital using retained tissue specimens from the Department of Oral Pathology and Microbiology over a period of eight months, from March 2024 to October 2024. The materials used for the study included formalin-fixed tissue specimens from departmental samples, alcohol solutions, xylene, kerosene, and cedarwood oil as clearing agents, paraffin wax for embedding, haematoxylin, eosin, acid alcohol, water, a Magnus binocular microscope, glass slides, coverslips, beakers, staining jars, forceps, gloves, and tissue-processing equipment, including a Leica microtome, slide-warming table, and hot air oven.

Study Procedure

The study was carried out in the following steps: Sample collection and preparation, Tissue processing and embedding, Tissue sectioning and slide preparation, Staining of tissue sections with H&E, Evaluation of H&E-stained sections.

A. Sample Collection and Preparation

Twenty formalin-fixed tissue specimens were obtained from the Department of Oral Pathology. Each specimen was sectioned into three equal pieces and assigned to one of three groups based on the clearing and deparaffinising agent used during tissue processing. Thus, the total number of study samples was 60 (N=60), with 20 specimens in each group (n=20):

Group A: Clearing and deparaffinising agent – xylene (20 specimens)

Group B: Clearing and deparaffinising agent - kerosene (20 specimens)

Group C: Clearing and deparaffinising agent - cedarwood oil (20 specimens)

B. Tissue Processing and Embedding

All specimens underwent routine tissue processing. Dehydration was performed using graded alcohols (50%-100%). Clearing was carried out using the respective clearing agents: xylene for Group A, kerosene for Group B, and cedarwood oil for Group C [Table/Fig-1] [11,12]. Following clearing, tissues were embedded in molten paraffin wax, and paraffin blocks were prepared.

C. Tissue Sectioning and Slide Preparation

Sections of 4 μm thickness were obtained using a semi-automatic rotary microtome (Leica, Germany). The sections were floated on a water bath, mounted on albumin-coated glass slides, and dried on a slide-warming table.

D. Staining of Tissue Sections with Haematoxylin and Eosin

The slides were deparaffinised using the same clearing agents employed during processing (Group A: xylene, Group B: kerosene, Group C: cedarwood oil), rehydrated through graded alcohols, and stained with Harris haematoxylin for 15 minutes. After bluing, cytoplasmic counterstaining was performed with eosin for two minutes [Table/Fig-2] [11,12]. The stained sections were then dehydrated, cleared, and mounted using Dibutylphthalate Polystyrene Xylene (DPX).

Steps	Group A (Control)	Group B (Experimental)	Group C (Experimental)
Dehydration			
50% Alcohol	1 hour	1 hour	1 hour
60% Alcohol	1 hour	1 hour	1 hour
70% Alcohol	1 hour	1 hour	1 hour
80% Alcohol	1 hour	1 hour	1 hour
90% Alcohol	1 hour	1 hour	1 hour
100% Alcohol - I	1 hour	1 hour	1 hour
100% Alcohol - II	1 hour	1 hour	1 hour
100% Alcohol - III	1 hour	1 hour	1 hour
Clearing agent			
Xylene I	1 hour	-	-
Xylene II	1 hour		-
Kerosene I	-	2 hours	-
Kerosene II	-	2 hours	-
Cedarwood oil	-	-	Over night

[Table/Fig-1]: Tissue processing procedure of control group and experimental groups [11,12].

Steps	Group A (control)	Group B (experimental)	Group C (experimental)
Deparaffinisation	Xylene I - 2min	Kerosene I- 20 min	Cedarwood oil-2min
	Xylene II - 2min	Kerosene II- 20 min	
Rehydration	100% alcohol - 1min	100% alcohol - 2min	100% alcohol - 1min
	90% alcohol- 1min	90% alcohol - 2min	90% alcohol- 1min
	70% alcohol- 1min	70% alcohol - 2min	70% alcohol- 1min
	50% alcohol- 1min	50% alcohol - 2min	50% alcohol- 1min
	Wash with water		
Nuclear staining	Hematoxylin -15 min		
	Wash with water		
Bluing	1% acid alcohol-1 min		
	Wash with water		
Cytoplasmic staining	Eosin - 2 min		
	Wash with water		
Dehydration	50% alcohol - 1 min	50% alcohol - 1min	50% alcohol - 1min
	70% alcohol- 1 min	70% alcohol- 1min	70% alcohol- 1min
	90% alcohol- 1 min	90% alcohol- 1min	90% alcohol- 1min
	100% alcohol- 1 min	100% alcohol- 1min	100% alcohol- 1min
Deparaffinisation	Xylene I - 2 min	Kerosene I- 20 min	Cedarwood oil-2min
	Xylene II - 2 min	Kerosene II- 20 min	
Mounting	DPX	DPX	DPX

[Table/Fig-2]: Steps involved in Haematoxylin and Eosin staining [11,12].

E. Evaluation of H&E-stained Slides

The H&E-stained sections were independently evaluated by two blinded observers using a binocular light microscope (Magnus, India) under 4×, 10×, and 40× magnifications. Parameters assessed included the quality of cytoplasmic staining, nuclear staining, and staining contrast. Scoring was assigned as follows: poor=1, fair=2, and good=3. Artifacts were evaluated and scored as presence of artifacts=1 and absence of artifacts=2 [10].

STATISTICAL ANALYSIS

The results were tabulated, and statistical analysis was performed using IBM SPSS version 20 software (IBM SPSS, IBM Corp., Armonk, NY, USA). Descriptive statistics, Fisher's exact test, Chi-square test, Kendall's tau-b, and Cohen's kappa statistics were used to analyse the data. A p-value of <0.05 was considered statistically significant.

RESULTS

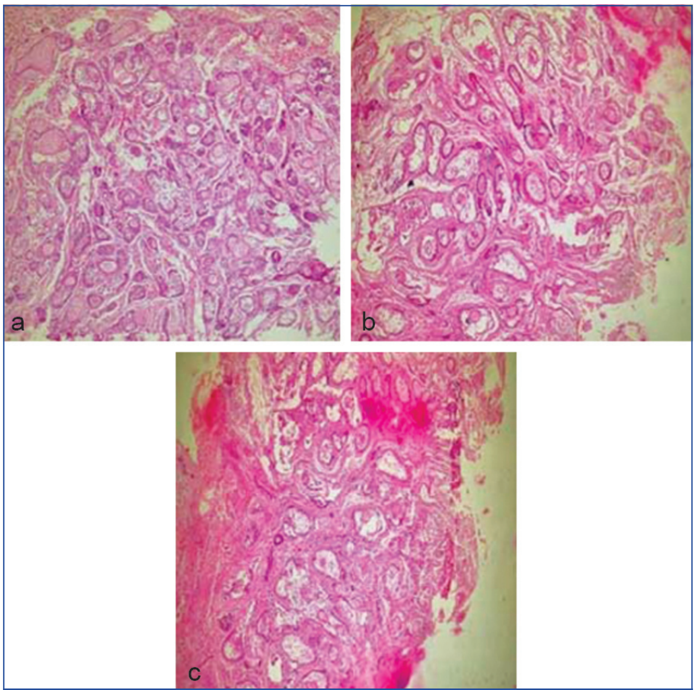
A total of 60 specimens, with 20 specimens in each group, were evaluated.

Cytoplasmic Staining

In Group A (xylene), 14 samples (70%) showed good cytoplasmic staining, while six samples (30%) showed fair staining quality. In Group B (kerosene), 10 samples (50%) showed good staining, and 10 samples (50%) showed fair quality. In Group C (cedarwood oil), 7 samples (35%) showed good staining, 12 samples (60%) showed fair quality, and 1 sample (5%) showed poor quality. However, the differences in cytoplasmic staining among the groups were not statistically significant (p=0.122) [Table/Fig-3,4].

Group	Cytoplasmic staining			Test statistic	P value
	Poor	Fair	Good		
Xylene	0	6	14	6.159	0.122
	0.0%	30.0%	70.0%		
Kerosene	0	10	10		
	0.0%	50.0%	50.0%		
Cedarwood oil	1	12	7		
	5.0%	60.0%	35.0%		

[Table/Fig-3]: Comparison of Cytoplasmic staining among the study groups.
Fisher's exact test; p≤0.05 considered statistically significant



[Table/Fig-4]: Cytoplasmic staining. Group A: Xylene; Group B: Kerosene; Group C-Cedarwood oil (H&E, 20X).

Nuclear Staining

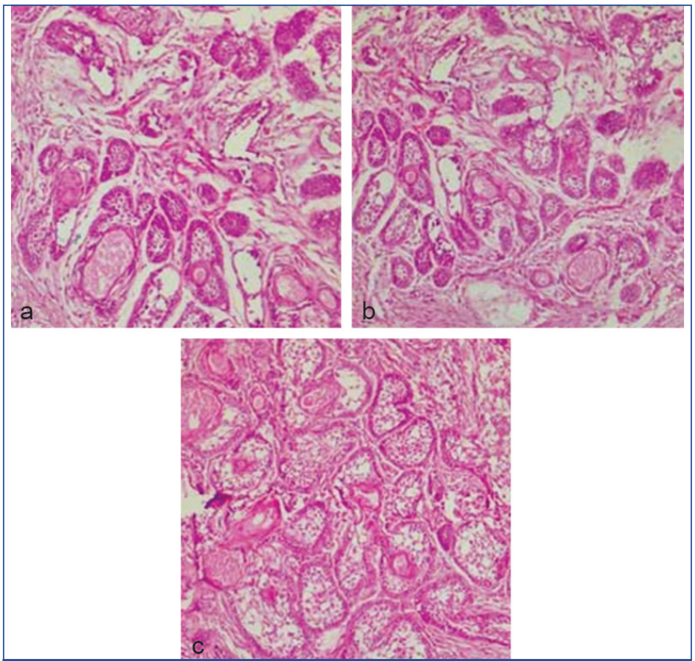
In Group A, 11 samples (55%) showed good nuclear staining, and 9 samples (45%) showed fair quality. In Group B, 7 samples (35%) showed good quality, 12 samples (60%) showed fair quality, and 1 sample (5%) showed poor nuclear staining. In Group C, 4 samples (20%) showed good quality, 15 samples (75%) showed fair quality, and one sample (5%) showed poor quality. The differences in nuclear staining among the groups were not statistically significant (p=0.127) [Table/Fig-5,6].

Staining Contrast

In Group A, 19 samples (95%) demonstrated good staining contrast, while 1 sample (5%) showed fair quality. In Group B, 7 samples (35%) showed good staining contrast, 10 samples (50%) showed fair quality, and 3 samples (15%) showed poor quality. In Group C, 19 samples (95%) showed good staining contrast, and 1 sample (5%) showed fair

Group	Nuclear staining			Test statistic	P value
	Poor	Fair	Good		
Xylene	0	9	11	5.95	0.127
	0	45.0%	55.0%		
Kerosene	1	12	7		
	5.0%	60.0%	35.0%		
Cedarwood oil	1	15	4		
	5.0%	75.0%	20.0%		

[Table/Fig-5]: Comparison of Nuclear staining among the study groups.
Fisher's exact test; p≤0.05 considered statistically significant



[Table/Fig-6]: Nuclear staining. Group A: Xylene; Group B: Kerosene; Group C: Cedarwood oil (H&E, 20X).

quality. The differences in staining contrast among the groups were statistically significant (p<0.001) [Table/Fig-7,8].

Artifacts

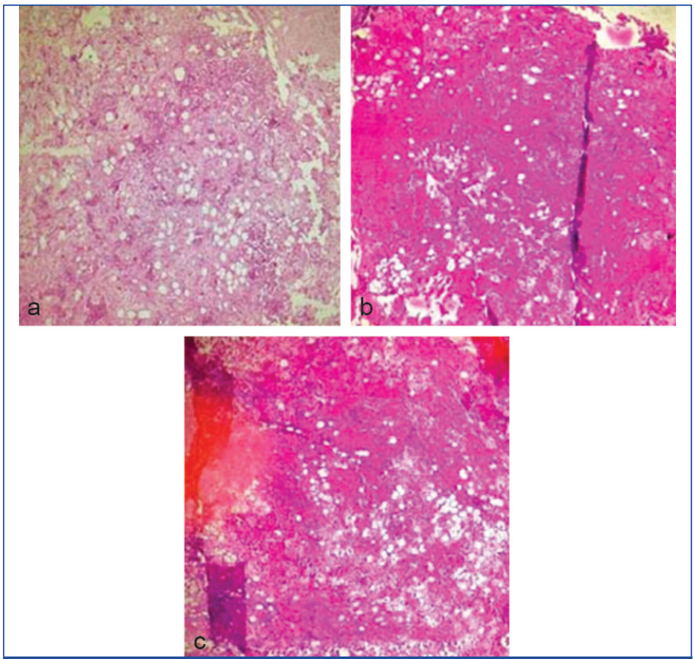
Artifacts were observed in 3 samples (15%) in Group A, 14 samples (70%) in Group B, and 4 samples (20%) in Group C. The differences in the presence of artifacts among the groups were statistically significant (p<0.002) [Table/Fig-9,10].

DISCUSSION

In the present study, the majority of samples in Group A processed with xylene exhibited good cytoplasmic and nuclear staining quality. Group B samples processed with kerosene showed an equal distribution of good and fair staining, whereas Group C samples processed with cedarwood oil predominantly demonstrated fair staining. However, the differences in staining performance among the groups were not statistically significant, suggesting that both kerosene and cedarwood oil are capable of producing acceptable cytoplasmic and nuclear staining.

Group	Staining contrast			Test statistic	P value
	Poor	Fair	Good		
Xylene	0	1	19	14.79	<0.001*
	0.0%	5%	95.0%		
Kerosene	3	10	7		
	15.0%	50.0%	35.0%		
Cedarwood oil	0	1	19		
	0.0%	5%	95.0%		

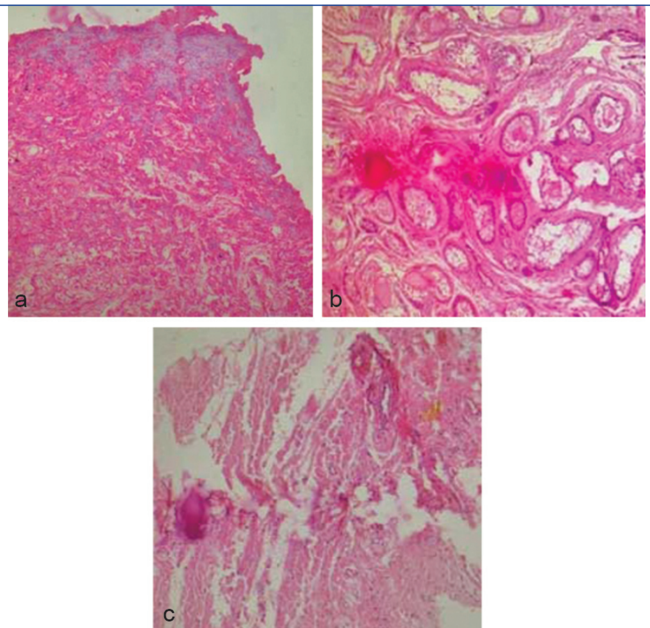
[Table/Fig-7]: Comparison of staining contrast among the study groups.
Fisher's exact test; p≤0.05 considered statistically significant; *denotes significance



[Table/Fig-8]: Staining Contrast. Group A: Xylene; Group B: Kerosene; Group C: Cedarwood oil (H&E, 20X).

Group	Artifacts		Test statistic	P value
	Present	Absent		
Xylene	3	17	12.99	0.002*
	15.0%	85.0%		
Kerosene	14	6		
	70.0%	30.0%		
Cedarwood oil	4	16		
	20.0%	80.0%		

[Table/Fig-9]: Comparison of artifacts among the study groups.
Chi square test; p≤0.05 considered statistically significant; * denotes significance



[Table/Fig-10]: Artifacts. Group A: Xylene; Group B: Kerosene; Group C: Cedarwood oil (H&E, 20X).

Studies by Dineshshankar J et al., (2019), Shah AA et al., (2017), and Singh P et al., (2023) reported that tissues processed and cleared with kerosene demonstrated comparable clearing and staining quality to xylene without significant alterations in tissue morphology or cellular details [10-12]. Similarly, studies by Thamilselvan S et al., (2021) and Viswasini RD et al., (2021) using cedarwood oil concluded that tissues processed with cedarwood oil showed high-quality staining with good preservation of tissue architecture [8,9]. The findings of the present study were partially consistent with these reports, as both kerosene and cedarwood oil demonstrated fair performance in cytoplasmic and nuclear staining when compared with xylene-processed tissues.

The excellent performance of xylene can be attributed to its ability to fulfill the essential requirements for optimal cytoplasmic and nuclear staining. Xylene is colorless and does not interfere with staining solutions, thereby enhancing the visibility of cytoplasmic details [4,5,7]. Its low viscosity and high miscibility-due to the presence of both polar and non-polar bonds in its chemical structure-with alcohol (polar hydrocarbon chains) and paraffin wax (non-polar hydrocarbon chains) allow for complete removal of alcohol from tissues. This facilitates efficient paraffin wax infiltration, rendering the tissue sufficiently firm to allow the cutting of thin sections. Consequently, staining becomes easier, resulting in optimal haematoxylin uptake and superior nuclear staining [4,7].

Furthermore, xylene has a refractive index of 1.49-1.51, which is comparable to that of tissue components and mounting media. This similarity imparts tissue transparency, allowing light to pass through effectively and improving visualisation of cytoplasmic and nuclear details. Additionally, xylene does not interfere with the dissolution of eosin, an aniline dye, thereby ensuring optimal cytoplasmic staining [4,7].

The fair performance of kerosene may be attributed to its low viscosity, which allows easy tissue penetration; however, its miscibility with alcohol is comparatively poor due to its predominantly nonpolar chemical structure and relatively high molecular weight. This limits its ability to completely remove alcohol and wax from tissues, thereby interfering with effective staining. Although the refractive index of kerosene (1.44) is reasonably compatible with microscopic media for light penetration, its light amber color further reduces the clarity and visibility of cytoplasmic staining [10-12].

The fair performance of cedarwood oil can be explained by the fact that, although its refractive index (1.48-1.52) closely matches that of tissue components and mounting media, its high density (approximately 1.01 g/mL), high viscosity, high molecular weight (222.37 g/mol), and reduced miscibility with other processing solutions hinder rapid tissue penetration. These properties result in incomplete clearing and deparaffinisation despite overnight immersion [8,9].

In the present study, the majority of Group A (xylene) and Group C (cedarwood oil) sections demonstrated good staining contrast, whereas Group B (kerosene) sections predominantly showed fair staining contrast. This difference was statistically significant, suggesting that cedarwood oil provides staining contrast comparable to xylene, whereas kerosene fails to achieve similar results. These findings were consistent with studies by Dineshshankar J et al., (2019), Shah AA et al., (2017), and Singh P et al., (2023) using kerosene [10-12], and by Indu S et al., (2014) and Shivani R et al., (2025) using cedarwood oil [13,14].

A crucial factor contributing to effective clearing is the refractive index of the clearing agent. Both xylene and cedarwood oil possess refractive indices very close to that of glass (approximately 1.51-1.52). The use of a clearing agent with an appropriate refractive index minimises light refraction and scattering as light passes through the glass slide, cleared tissue, mounting medium, and microscope lens. This enhances tissue transparency and enables clear visualisation of stained cellular structures. Additionally, both xylene and cedarwood oil share key physical (colorless) and chemical (solvent) properties that make them effective clearing agents in histopathology [12-14].

The fair staining contrast observed in kerosene-processed tissues may be due to incomplete deparaffinisation, leading to reduced dye binding compared to cedarwood oil. Furthermore, kerosene-processed slides exhibited a pale yellowish or bluish tint, which diminished contrast owing to the inherent color of kerosene [8-10].

The present study demonstrated statistically significant differences in the presence of artifacts among all groups, with artifacts being least frequent in the xylene group. This observation is consistent with the findings of Chaudhuri D et al., (2023) [4]. The superior performance of xylene may be attributed to its efficient clearing and deparaffinising capacity, absence of impurities, neutral pH, optimal ionic strength, and compatibility with histological dyes [4].

Tissues processed with kerosene exhibited the highest incidence of artifacts (75%), primarily related to processing and staining. In contrast, cedarwood oil–processed slides did not show a pale yellowish tint and were mostly clear with good contrast. These observations align with studies by Dineshshankar J et al., (2019), Shah AA et al., (2017), and Singh P et al., (2023) for kerosene [10-12], and Indu S et al., (2014) and Shivani R et al., (2025) for cedarwood oil [13,14].

The high artifact rate in kerosene-processed tissues may be attributed to its composition, as kerosene is a mixture of hydrocarbons such as paraffin and naphthenes and contains multiple impurities that can precipitate stains. Many slides also exhibited a hazy appearance, likely due to residual wax resulting from incomplete deparaffinisation [10-12]. Reduced clarity may also be related to the pale yellow color of kerosene. Poorly stained or weakly stained areas may result from inadequate clearing, leading to retention of dehydrating agents within the tissue. When these residual agents interact with paraffin wax and staining solutions, they inhibit stain uptake, resulting in uneven or weak staining. Additionally, kerosene-processed tissues appeared harder than xylene-processed tissues, possibly due to pH-related factors, as kerosene lacks defined acidic or alkaline properties [10-12].

Tissues processed with cedarwood oil exhibited 20% artifacts related to processing and staining. The primary limitation of cedarwood oil is its high molecular weight and viscosity, which slow tissue penetration and may result in incomplete clearing and deparaffinisation [9,14]. Although prolonged immersion in cedarwood oil has been reported to reduce artifacts, it is not recommended due to the risk of tissue over-hardening and loss of morphology [1,2]. Residual wax observed in both kerosene- and cedarwood oil-processed sections may result in incomplete or partial staining, leading to the "pink disease" artifact, as described by Nedzel GA (1951) [15].

Overall, the present study demonstrated comparable clearing performance of kerosene and cedarwood oil when compared with xylene. However, cedarwood oil showed superior performance as a deparaffinising agent compared to kerosene, although xylene remained the gold standard.

Limitation(s)

The present study was conducted on a limited number of retained tissue specimens, which may affect the generalisability of the results. Assessment of staining quality and artifacts was subjective and dependent on observer evaluation, which may introduce bias. Additionally, the long-term effects of kerosene and cedarwood oil on tissue integrity and diagnostic accuracy were not evaluated and warrant further investigation.

CONCLUSION(S)

The present study concluded that xylene remains the optimal clearing and deparaffinising agent for routine histopathology, provided that adequate safety measures are implemented to minimise toxic exposure. Kerosene alone is inadequate for high-quality routine H&E staining and tissue processing; however, its efficacy may be improved by refining and removing impurities, making it a cost-effective alternative. Cedarwood oil may serve as a safer substitute for xylene in modern histopathology laboratories, although its high cost limits routine use. The performance of cedarwood oil could be further enhanced by reducing its viscosity to facilitate better tissue penetration.

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